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mTOR Signaling Pathway Genes Effect in COVID-19 Infection

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ABSTRACT: Coronavirus disease 2019 (Covid-19) is an infectious disease that causes severe acute respiratory illness caused by coronavirus 2 (SARS-CoV-2). SARS-CoV-2 uses host-specific metabolic pathways, including mTOR. The mTOR pathway is hyperactive in viral respiratory tract infections and contributes positively to viral replication. 100 samples were evaluated, 50 patients (Female=23, Male=27), and 50 controls (Female=29, Male=21). The patients were individuals who were COVID-19 positive. We detected expression changes of 5 genes in mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (*MLST8*, *mTOR*, *RPTOR*, *MAPKAP1*, and *RICTOR*). Serum samples were obtained from all patients. The expression changes of mTORC1 and mTORC2 Complex genes were evaluated with the Real-time PCR method. Receiver operating curve (ROC) analysis was performed to define the diagnostic power of these genes. Expression changes of five genes in the mTORC1 and mTORC2 complex were statistically significant (p =0.001) and upregulated in serum. The area under the ROC Curve values indicating the diagnostic power of genes were 0.948, 0.771, 0.851, 0.798, and 0.805, respectively. The increased expression of these genes in the mTOR pathway used by SARS-CoV-2 in viral replication during the infection process shows that these genes and protein products are candidates for treatment targets. At the same time, the high discriminative power of these genes in patients from controls indicates their diagnostic potential in serum samples.

Keywords: *COVID-19*, *SARS-CoV-2*, *mTOR complex 1 (mTORC1)*, *mTOR complex 2 (mTORC2)*

1 INTRODUCTION

Coronavirus is a virus from the Coronaviridae family [1]. The virus encodes four main structural proteins: Spike (S), Membrane (M), Envelope (E), and Nucleocapsid (N) [2]. The S protein is a type I transmembrane protein expressed on the virus surface and is responsible for the virus binding to its receptor in the target cell and entry into the cell [3]. S protein binds to receptors of coronavirus, and its ability to induce neutralizing antibodies in vivo has been reported [4]. The coronavirus infects mammals and birds, causing respiratory illness and symptoms such as diarrhea [5].

Severe acute respiratory syndrome (SARS) infection is potentially fatal in humans [6]. SARS infection is a disease that occurs with shortness of breath and high fever that

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turns into pneumonia [7]. The emergence of Covid-19 disease, caused by the new type of coronavirus called SARS-CoV-2, has posed a pandemic threat to public health. Covid-19 infection is a novel coronavirus causing 2,073,361 confirmed deaths worldwide by August 2022 (https://www.who.int/europe/emergencies/situations/covid-19). Therefore, researchers began to investigate the pathophysiology of the disease caused by the virus to uncover possible treatment methods for this disease and to identify therapeutic agents that may be effective against the disease.

Recent studies have focused on signaling pathways. One of these pathways, the mTOR signaling pathway, is involved in the basic cellular processes of transcription, protein synthesis, and cell metabolism [8]. The mTOR signaling pathway is regulated by interferons as part of the anti-viral response during viral infections [9]. In vitro, studies have shown that kinase inhibitors target the mTOR signaling pathway and reduce the spread of MERS-COV [10]. In addition, these studies suggest that the mTOR signaling pathway may be a potential drug target.

In this study, which we planned from this point of view, we quantitatively evaluated the changes in the expression of five genes in the mTOR signaling pathway in Covid-19 patients. We aimed to detect genes with changes in their activities and to evaluate their diagnostic power. Thus, it will contribute to the elucidation of the molecular mechanisms related to the pathophysiology of Covid-19 disease. In addition, the use of mTOR inhibitors in the treatment of Covid-19 disease will come into prominence. The use of a drug that developed against coronavirus, an RNA virus, for other RNA viruses may come into question.

2 MATERIAL AND METHOD

Population data and clinical epidemiology

The study group consisted of 50 patients and 50 healthy control individuals who applied to Malatya Turgut Ozal University, Faculty of Medicine, Department of Chest Diseases. The subjects have given their written informed consent.

Typically, patients have a fever, shortness of breath, cough, weakness, pain, dizziness, nausea, headache, vomiting, anorexia, chest pain, and back pain. The patients applied to the clinic for one, several, or more of these clinical histories. Patients were diagnosed with COVID-19 tests compatible with CT (Computed tomography) and diagnosed (they were experiencing the disease for the first time). Oxygen saturation was between 87-98. Oxygen therapy has been given by nasal, reservoir, intubated, nasal oxygen + chamber, reservoir + CPAP, and mask. Diseases that accompany the COVID-19 infection were seen as ischemic cerebrovascular disease (CVD) and

hypertension (HT), diabetes mellitus (DM) and immune tolerance, congenital adrenal hyperplasia (CAH), chronic renal failure (CRF), hemorrhagic CVD, ischemic CVD, asthma, pneumonia, ankylosing spondylitis, acute kidney failure (ARA), chronic obstructive pulmonary disease (COPD), subarachnoid hemorrhage (SH), schizophrenia, and asthma. Control subjects were PCR-negative with a clinical history similar to COVID-19-positive patients. The whole peripheral blood samples (5 ml) from patients and healthy controls were collected in gel tubes, centrifuged at +4 °C, and serum samples were stored at -80 °C.

Inclusion and exclusion criteria

Fever-decreased lymphocyte count, early-stage chest radiology and ground-glass opacities, bilateral inflammation, and epidemiological history were among the inclusion criteria. In addition, upper respiratory tract specimens, including nasopharyngeal, oropharyngeal, and nasal swabs were collected, and these materials were part of routine diagnostics to detect SARS-CoV-2 [11]. Individuals who did not have acute fever (>37.5°C) in the last 72 hours and had typical lung imaging were confirmed by repeated measurements and were excluded from the patient group. Clinical data and rapid deaths identified in the light of the data obtained during the pandemic period required urgent diagnosis and production of treatment protocols. For this reason, the boundaries of the disease were drawn based on the symptoms included in the inclusion criteria, since it is not a condition that has been encountered before and even if it is encountered, it does not result in sudden deaths [12]. With diagnostic tests entering the urgent phase, making a PCR diagnosis and starting treatment immediately became the best option. The difficulty in obtaining diagnostic kits forced clinicians to select patients with a preliminary diagnosis. As a result, patients who met the inclusion criteria were diagnosed with PCR and treated. In this context, individuals who applied to the hospital with these complaints had appropriate epidemiology and radiological findings were included in the study group.

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Why expression of mTOR complex genes

SARS-CoV-2 uses a host's metabolic pathway, such as mTOR, to synthesize virus particles. mTOR is an important treatment target because hyperactivation of the mTOR pathway contributes positively to viral replication. Investigation of the expression changes of mTORC1 and mTORC2 complex genes in our study will contribute to the evaluation of the infection process.

Determination of SARS-CoV-2

Many diagnostic kits were used to diagnose the disease during the pandemic process. Although there are still debates about their accuracy and effectiveness, kits that received FDA approval have been put into use. The important point in this study is that while these diagnostic tests are carried out rapidly in each region, it is important for this region as it allows the test results to be compared with others and is evidence for the success of the diagnosis. The diagnostic kit used for our patient group representing a region in Turkey is BIO-SPEEDY® DOUBLE GENE RT-QPCR KIT (BIO-SPEEDY, BS-SY-WCOR-307-250, bioeksen, Hindenburgstrasse, Germany). The feature of the kit is that it provides simultaneous detection of SARS-CoV-2 and Variants of Concern (VOC) (Alpha, Beta, Gamma, and Delta) in a single reaction. The test includes real-time, one-step reverse transcription PCR used for the qualitative detection of SARS-CoV-2-specific open reading frame 1 ab (Orf1ab), nucleocapsid (N), and human *ribonuclease P (*RNase P) gene fragments. There were occasional access problems for the modified kits, which are among the urgent solutions for pre-treatment diagnosis on a country basis, and they did not have the chance to be selected. Research on this kit, which is among the kits available and used in a regional hospital in Turkey, showed that this
kit and other kits are similar in sensitivity and specificity [13].

RNA extraction and quantification

A total of 5 mL of *blood* was *drawn into* a serum *gel tube.* Total RNA was extracted from serum by RNeasy Mini Kit (Qiagen, Hilden, Germany) according to commercial protocol. RNA purity and quantification were performed with a spectrophotometer (MaestroNano Micro-Volume Spectrophotometer, MN-913, Maestrogen, Taiwan). The amount and purity of RNA from each sample were determined spectrophotometrically using a measuring range of 2~2000 ng/µL. 0.8 U of DNase I per 1 µg of RNA was used to purify RNA from DNA according to the kit protocol (Thermo Scientific™, EN0521, Waltham, MA USA) and incubated at 37°C for 30 minutes.

Complementary DNA (cDNA) synthesis

Complementary DNA was performed using an ABScript III RT Master Mix for qPCR Kit (ABclonal, United Kingdom). Reverse transcription (RT) reactions were prepared as 4µl 5xABScript III RT Mix, up to 1 ug total RNA, Nuclease-Free Water in a total of 20µl reaction. The Reactions were placed in an automated Gene Amp PCR System 9700 (Applied Biosystems, ABD) and incubated at 55°C for 15 minutes and at 85°C for 5 minutes. At the end of the period, they were immediately placed on ice and stored at -20 °C until further analysis.

Quantitative-Comparative CT (cycle thresold) (ΔΔCT) Real-time PCR (qPCR) analysis

Primers were synthesized (Synbio Technologies, USA) using SYBR Green probe technology. ACTB Human-Forward Sequence: 5′-CACCATTGGCAATGAGCGGTTC-3′ and ACTB Human-Reverse Sequence: 5′-AGGTCTTTGCGGATGTCCACGT-3′.
MLST8 Human- Forward Sequence: 5′-CAGGTGAATGCCTTGGAGGTCA-3′ and MLST8 Human- Reverse Sequence: 5′-AGTCGATGCTGCCGCAGTTGTT-3′,
mTOR Human-Forward Sequence: 5′AGCATCGGATGCTTAGGAGTGG-3′ and mTOR Human-Reverse Sequence: 5′-CAGCCAGTCATCTTTGGAGACC-3′,
RPTOR Human-Forward Sequence: 5′-GATCGTCAACAGCTATCACACGG-3′ and RPTOR Human-Reverse Sequence: 5′-CGAGTCGAAGTTCTGCCAGATC-3′,
RICTOR Human-Forward Sequence: 5′-GCCAAACAGCTCACGGTTGTAG-3′ and RICTOR Human-Reverse Sequence: 5′-GTCACCGAGTTACGAAGTAGACC-3′,
MAPKAP1 Human-Forward Sequence: 5′-CAGGACAGACTGCTGCCAATGA-3′ and MAPKAP1 Human-Reverse Sequence: 5′-CTGTTACAGTCACGGATGACGG-3′.
Real-time PCR mix was prepared as 2X SYBR Green reaction mix (ELK Biotechnology, China), 10 µM forward and

reverse primer, ddH2O, and 2 µl cDNA in total 10 µl reaction. RT-PCR methods were performed using a Real-Time PCR system RotorGeneQ (Qiagen, Hilden, Germany). Thermal cycling conditions were 30 seconds at 95°C followed by 40 cycles at 95°C for 5 seconds, 50-60°C for 30 seconds, and 72°C for 30 seconds. Melting curve stage added. All reactions were run in duplicate, and samples were normalized using the expression of the beta-actin (*ACTB*) housekeeping gene. Relative quantification of normalized samples was calculated according to the formula $2^{-\Delta\Delta Ct}$ and given as fold change value [14].

3 STATISTICAL ANALYSIS

According to the power analysis, the number of subjects in each group was calculated as 50 for a 0.20 difference between the two groups to be significant (Type I error = 0.05, power of the test = 0.8). Normality analyzes were performed with Kolmogorov-Smirnov and Shapiro-Wilk tests. ANOVA test was used for comparison between and within groups. Mann-Whitney U test was used to analyze the differences between groups. The Kruskal-Wallis test was used to measure the differences in gene expressions. Differences in mRNA were determined using the Bonferroni-corrected one-way ANOVA test for multiple comparisons at the $\alpha = 0.05$ cutoff point. The diagnostic values of the genes were evaluated by receiver operating characteristics (ROC) analysis. The diagnostic power of the genes was made according to Hosmer's rating [15]. In addition, logistic regression analysis was performed to determine the diagnostic values of these genes. Statistically, $p < 0.05$ was considered significant. Data were analyzed using the SPSS statistical package for Windows (Version 22.0, Armonk, NY: IBM Corp).

ROC analysis for mRNA diagnostic values

The ROC curve is a graphical representation of the relationship between sensitivity and selectivity. ROC analysis is an analysis method that contributes significantly to the clinical decision-making process. The area under the ROC curve determines the accuracy of the test in distinguishing patients from healthy controls, and the size of the area under the curve also indicates the statistical significance of the discrimination ability of the diagnostic test studied.

4 RESULTS

Clinical characteristics

The age and gender characteristics of the study population are given in Table 1. The number of males and females in the groups was approximately equal. Age and gender distribution in the groups are given in Figure 1. The mean age was 58.60 in the patient male, 66.13 in the patient female, 59.43 in the healthy male, and 55.28 in the healthy female. In the analysis of age distribution, 71 and 69 years were higher in the control and patient groups. In the multiple and comparative

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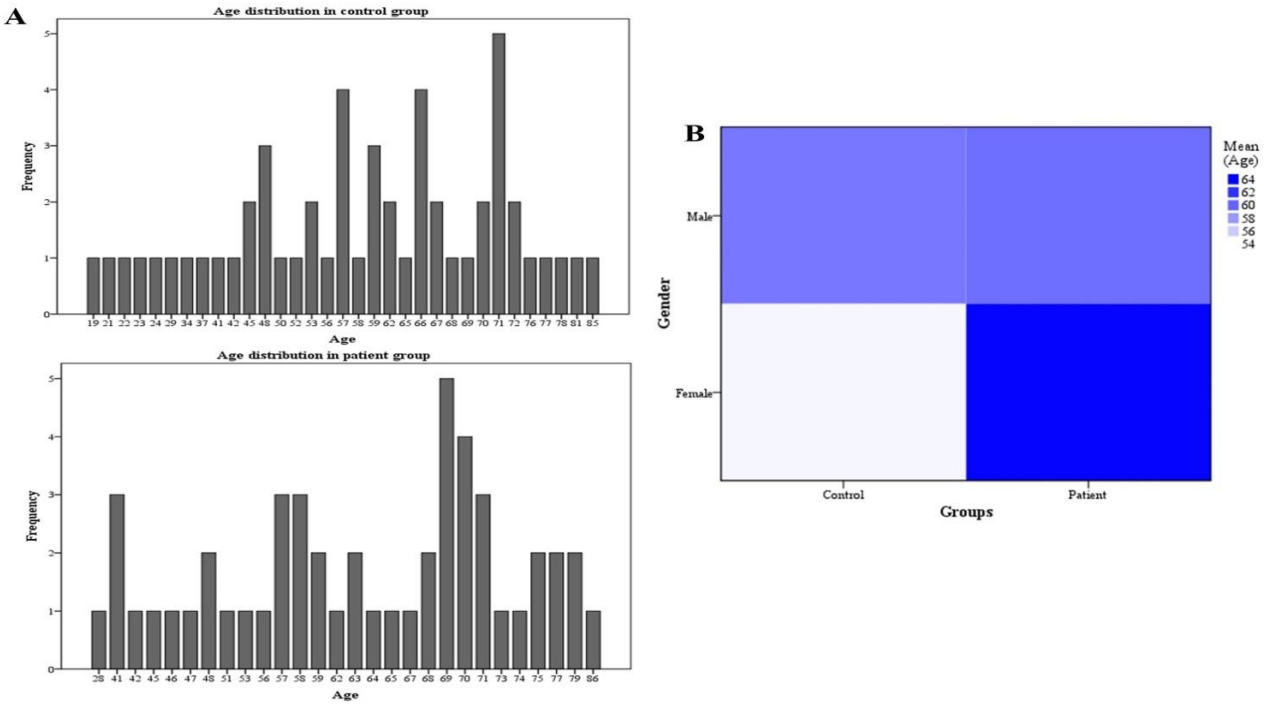

Figure 1. Analysis of age and gender factor in groups. In the age distribution analysis, it was observed that the 69-70 age group was high in the control and patient groups. Data are presented in groups as frequency and heat maps. **A.** Frequency representation of age distribution in the control and patient groups. **B.** Representation of age and gender distribution in the control and patient groups as means.

analyses performed, there was no statistical significance for age (p = 0.091) and gender (p = 0.234). Gene expressions were not associated with age and gender (p >0.05).

SARS-CoV-2 types our patient's groups.

Table 1. The characteristics of the two cohorts.

Covariates	Control (n)* (%)	Patient (n)** (%)
Age (years)	19-85	28-86
Gender		
Female	29 (58)	23 (46)
Male	21 (42)	27 (54)

*n = 50, **n = 50. NA; not applicable.

Pango lineage was expressed based on WHO records since they were samples according to the classification made at the beginning of the pandemic (https://www.who.int/publications/m/item/historical-working-definitions-and-primary-actions-for-sars- cov-2-variants). According to this (GSAID; Global Initiative on Sharing All Influenza Data);

Alpha ; Pango lineage-B.1.1.7, GISAID clade-GRY, nexstrain clade-20I (V1).

Beta; Pango lineage- B.1.351, GISAID clade-GH/501Y.V2, nexstrain clade- 20H (V2)

Gamma; Pango lineage- P.1, GISAID clade-GR/501Y.V3, nexstrain clade- 20J (V3)

Delta: Pango lineage- B.1.617.2, GISAID clade- G/478K.V1, nexstrain clade- 21A, 21I, 21J.

In the Omicron variant and subsequent classifications, Orf1ab and N positive mutations were determined as stated below (According to kit diagnostic targets).

BF.7; N: G30-, S33F

BQ.1; ORF1a: Q556K, L3829F, ORF1b: Y264H, N: E136D

BA.2.75 and CH.1.1; ORF1a: S1221L, P1640S, N4060S, ORF1b: G662S

XBB; ORF1a: K47R, ORF1b: G662S, S959P

Gene expression analysis

The expression analyses of the five genes belonging to the mTORC1 and mTORC2 complexes in COVID-19 patients and healthy individuals were found to be statistically significant ($p < 0.05$) (Table 2). In the descriptive statistical analyses performed to reveal the difference between COVID-19 patients and healthy control individuals, upregulation of five genes was detected in the patient group (Figure 2). We evaluated the contribution of these genes to Covid-19 disease by logistic regression analysis. All genes were modeled and analyzed. *mTOR* (OR = 1.088, 95% CI = 0.802-1.475, p = 0.001), *RPTOR* (OR = 1.154, 95% CI = 0.614-2.168, p= 0.001), *MAPKAP1* (OR = 1.160, 95% CI = 0.900-1.494, p=0 .001), *RICTOR* (OR = 0.991, 95% CI = 0.574-1.709, p = 0.001), and *MLTS8* was significant (OR = 2.288, 95% CI = 1.531-3.417, p =0 .0001).

ROC analysis for diagnostic values of five genes

The diagnostic value of all genes was graded high and statistically significant (Table 3) (Figure 3). According to the classification showing the diagnostic value of AUC values,[16] *MLST8* was found as "excellent" *RPTOR, RICTOR* "very good", *mTOR*, and *MAPKAP1* "good" (Table 4).

Table 2. Gene expression analysis five gene in case-control cohort (ANOVA).

Genes	Groups	N	Mean±SD	SE	95% CI		*P-value*
MLST8	Control	50	-4.446±2.164	0.306	-5.061	-3.830	**<0.001***
	Patient	50	1.416±2.837	0.401	0.610	2.222	
mTOR	Control	50	0.474±2.476	0.350	-0.230	1.177	**<0.001***
	Patient	50	3.413±3.239	0.458	2.492	4.333	
RPTOR	Control	50	-16.117±1.625	0.230	-16.579	-15.655	**<0.001***
	Patient	50	-13.359±2.141	0.303	-13.968	-12.750	
MAPKAP1	Control	50	0.322±3.046	0.431	-0.544	1.187	**<0.001***
	Patient	50	4.276±3.834	0.542	3.186	5.365	
RICTOR	Control	50	-1.578±1.837	0.260	-2.100	-1.055	**<0.001***
	Patient	50	0.970±2.403	0.340	0.287	1.653	

*P < 0.05 is significant. SD; standart deviation, SE; standart error, CI; confidence interval.

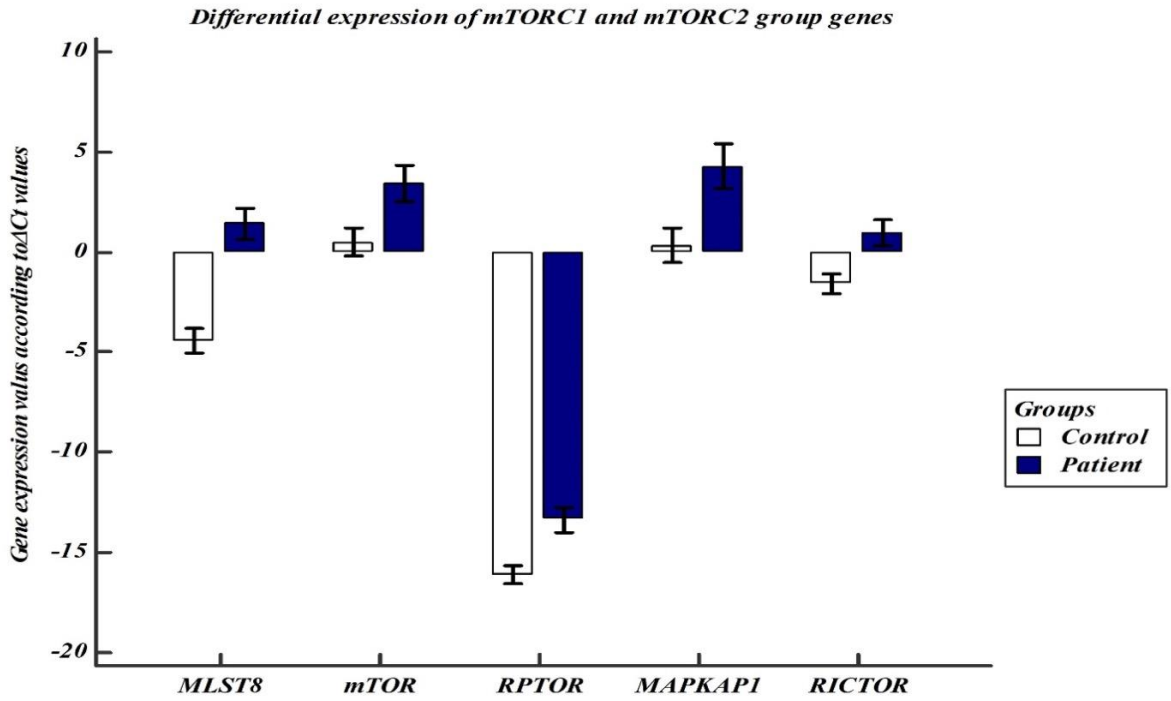

Figure 2. Differential gene expression analysis of five gene. The expression difference between COVID-19 patients and control subjects is presented in the graph. Expression values indicate the ΔCt. A Mann-Whitney U test was used to reveal the differences in miRNA expressions between groups. As a result of the analysis, a statistically significant difference was found between expressions and groups for five genes ($P < 0.001$).

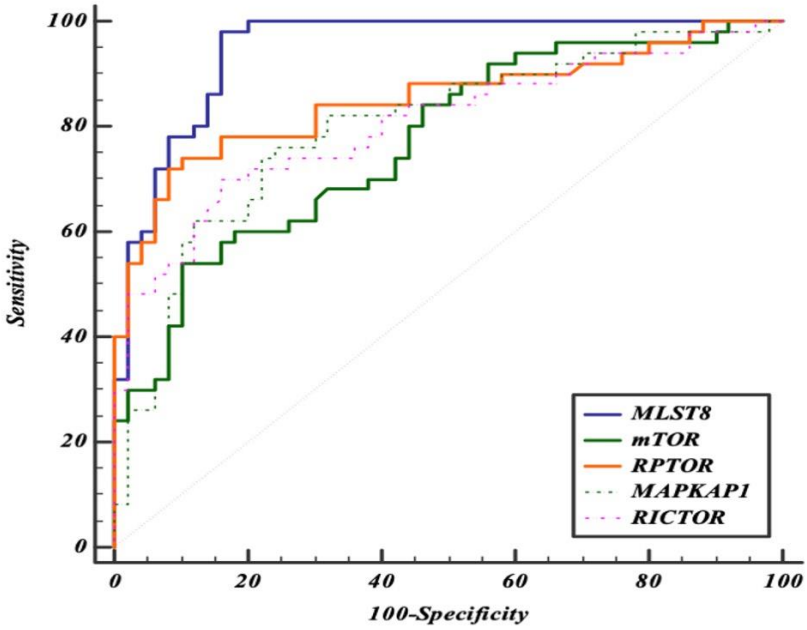

Figure 3. Roc curve analysis for diagnostic values of five genes. All AUC values were statistically significant ($p<0.001$).

Table 3. ROC curve analysis of five genes.

Compared Groups	Genes	AUC	95% CI	SE	P-Value	Specificity	Sensitivity	Criterion
Patient vs Control	*MLST8*	0.948	0.907-0.989	0.021	< 0.001	0.98	0.84	>-2.460
	mTOR	0.771	0.680-0.861	0.046	< 0.001	0.90	0.54	>3.255
	RPTOR	0.851	0.772-0.929	0.040	< 0.001	0.90	0.74	>-14.425
	MAPKAP1	0.798	0.710-0.886	0.045	< 0.001	0.76	0.76	>1.915
	RICTOR	0.805	0.718-0.892	0.044	< 0.001	0.84	0.70	>0.05

Table 4. Pairwase comparison of ROC curves.

Gene combinations	DBA	SE	95% CI	Z statistics	*P*-Value
MLST8 ~ mTOR	0.177	0.045	0.090-0.265	3.974	**0.000***
MLST8 ~ RPTOR	0.097	0.042	0.014-0.180	2.300	**0.022***
MLST8 ~ MAPKAP1	0.150	0.047	0.058-0.242	3.186	**0.001***
MLST8 ~ RICTOR	0.143	0.046	0.052-0.233	3.109	**0.001***
mTOR ~ RPTOR	0.080	0.049	-0.008-0.168	1.786	0.074
mTOR ~ MAPKAP1	0.027	0.066	-0.102-0.157	0.415	0.678
mTOR ~ RICTOR	0.034	0.056	-0.075-0.144	0.615	0.539
RPTOR ~ MAPKAP1	0.052	0.052	-0.049-0.154	1.019	0.308
RPTOR ~ RICTOR	0.045	0.031	-0.014-0.105	1.496	0.135
MAPKAP1 ~ RICTOR	0.007	0.046	-0.082-0.097	0.153	0.878

* $P < 0.05$ is statistically significant. SE; standard error, CI; confidence interval, DBA; the difference between areas.

5 DISCUSSION

COVID-19 has captured the world for a long time and has become a permanent disease due to its severe clinical symptoms and rapid spread. We don't know exactly how our immune system responds to Covid-19. Personal differences and underlying illnesses show that the process is individual. The vaccine and vaccination process, which cured the long-lasting COVID-19 disease in a short time, caused relief. In this process, much data was obtained for both diagnosis and treatment. However, several factors need to be identified to manage the disease. At this point, one of the most appropriate approaches would be studies to determine the markers and targets that can determine the genetic basis for this disease, whose course varies according to individual differences.

The most important reaction of COVID-19 is the severe inflammatory response seen in patients after infection and the cytokine storm in end-stage cases. Cytokine storm results in acute respiratory distress syndrome, multiple organ failure accompanied by lung and other organs, leading to death with progressive COVID-19. Activation of inflammatory pathways is the main factor here

[17]. Viral entry of the spike protein on the surface of the virus involves binding to host cell receptors and viral replication. As a result of the activation of the mTOR pathway by the Warburg effect, COVID-19 replication and inflammatory response are promoted. Thus, viral protein synthesis increases and cytokine production is forced. Therefore, mTOR is an important therapeutic target in COVID-19.

The virus uses a host metabolic mTOR pathway for particle synthesis. The reason why the virus targets a powerful pathway such as mTOR is that this signaling pathway is involved in mRNA translation [18]. The source of this power of the mTOR pathway is its ability to control metabolic events such as autophagy, cell proliferation, protein synthesis, cellular growth, proliferation, insulin, and response to oxygen [10]. This situation shows us the importance of the mTOR pathway in respiratory failure observed in COVID-19 patients and the presence of covariant diseases such as hypertension, cardiovascular disease, and diabetes. Clinical studies also show hyperactivation of the mTOR pathway during infection [19]. Investigating genes in the mTOR pathway, which has a significant role in COVID-19, may be a target in diagnosis and treatment. Rapamycin is the best-known mTORC1 inhibitor [20]. More potential targets need exploring to find durable solutions. mTOR has two protein complexes, mTORC1 and mTORC2 [8]. In both complexes, mTOR is the central protein. The mTORC1 complex includes mTOR, DEPTOR, PRAS40, RPTOR, FKBP12 rapa, and MLST8. RPTOR targets HEAT (Huntingtin, elongation factor 3, the A subunit of protein phosphatase 2A (PP2A), and the signaling kinase TOR1)[21] (usually involved in intracellular transport systems)[22] repeats while MLST8 targets the kinase active site. The mTORC2 complex includes DEP domain-containing mTOR-interacting protein (DEPTOR), Protor1/2, mSin1 (MAPKAP1), RICTOR, and MLST8. RICTOR targets HEAT repeats, while MLST8 targets the kinase active site.

Sirolimus, everolimus, and metformin are the detected inhibitors of the mTOR complex, and with this inhibition, cell cycle progression, transcription, translation, and protein synthesis are targeted [23]. mTOR complex of genes may be a new target for diagnosis and treatment in patients with Covid-19. Gene expression studies for COVID-19 refer to dysregulated regulation of various genes. Moni et al. compared SARS-CoV-2 with SARS-CoV, MERS-CoV, and influenza A strains H1N1, H3N2, and H5N1 in their genomic and transcriptomic analyses. They detected a striking upregulation for 40 SARS-CoV-2 infection response genes in patients' peripheral blood samples compared to other viruses [24]. Comorbidities with a disease can distort results and hinder the purification of the

target. Excluding comorbidities, multi-omic analyses using artificial intelligence in young patients detected up-regulation of metalloprotease and ADAM9 among genes with different expressive expressions [25]. The planned ex vivo study showed that inhibition of ADAM9 reduces SARS-CoV-2 uptake and replication in human lung epithelial cells. In a study investigating the reason for the low risk of Kovid-19 in children compared to adults, it was stated that the angiotensin-converting enzyme 2 (ACE2) receptor, which SARS-CoV-2 uses when entering the host, is expressed differently [26]. In a cohort study of 305 individuals aged 4 to 60 years, 49.8% of individuals had asthma. The result was that ACE2 expression was lowest in young children and higher with increasing age. It can be said that the effects of Covid-19 symptoms in the middle-aged group increase depending on certain genes. With the contribution of comorbid factors in advanced ages, the patient's condition gets worse and may result in death. Differential gene expression analyses of COVID-19 severity showed differential expression of 55 genes according to disease severity [27].

The *Orf 1ab*, *N*, and *Rnase P* gene regions are important targets in the viral process. The relationship between positive mutations in these targets and mTOR in patients was demonstrated for the first time in this study. Previous data report the association of mTOR and autophagy pathways with disease severity [28]. Additionally, increased mTOR signaling and comorbidities that pose a higher risk of mortality due to Sars-CoV-2 are common [29]. The mTOR pathway was suggested among the drug targets identified in interactome studies for Sars-CoV-2 [30]. The relationship of mTOR with autophagy is known. Disruption of autophagic circulation in cells infected with SARS-CoV-2 causes increased virus replication [31]. These results may be associated with serious symptoms of COVID-19.

Studies indicate that the expression of genes in the mTOR complex has a high effect potential in COVID-19 patients. Indeed, as we demonstrated in our study, *MLST8*, *mTOR*, *RPTOR*, *MAPKAP1*, and *RICTOR* genes were upregulated in our patient group with different comorbidities. Consistent with other studies, the hyperactivity of the mTOR pathway in COVID-19 is due to overexpression of the responsible proteins. mTOR pathway activation contributes to viral replication. It is currently a holistic solution to combating disease, as treatments are based on inhibiting the mTOR complex or PI3K or AKT. Targeting genes in the complex may be a more specific and effective approach. Epigenetics or phytotherapeutics can also offer effective solutions for this. It can also reduce the extra burden of drug side effects and comorbidities. The contribution of gene expressions to the

disease process is the first pillar of the research, and the second pillar is the diagnostic potential. Diagnostic analyses demonstrated that *MLST8* has the potential as a marker to distinguish patients from controls. *RPTOR* and *RICTOR*, are in second place, followed by *MAPKAP* and *mTOR* detected as significant markers. Age, gender, and comorbidities are not significant, but since the age range studied is young and old populations. In studies conducted for age and gender in COVID-19, it was seen that the mortality rate was higher in male patients and the risk was higher due to the added comorbidities with advancing age (i.e., diabetes, hypertension, cardiovascular disease, etc.) [32, 33].

6 CONCLUSIONS

In this study, we present information on the activity of five genes in the mTOR pathway in the cellular response to SARS-CoV-2. Additional biochemical and clinical studies are needed to demonstrate the role of mTOR inhibitors and modulators in treating COVID-19.

7 FUNDING

This work was supported by the Scientific Research Projects Unit of Malatya Turgut Ozal University (Project No:2021/7).

8 CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

9 ETHICAL APPROVAL

This study has been approved by the clinical research ethics committee of Malatya Turgut Ozal University (2021/27).

10 AUTHORS CONTRIBUTIONS

M.P. and S.G.Y. designed the study; M.P and E.S.T conducted experimental studies; A.G., L.A.D, D.A.O and M.O. collected patient data; M.P. and S.G.Y. prepared the initial manuscript; M.P. and S.G.Y. edited the initial version of the manuscript; all authors approved the final version of the manuscript.

11 REFERENCES

[1] De Groot RJ, Baker SC, Baric R, Enjuanes L, et al. Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses. *Elsevier*, 2011: 806-828.

[2] Troyano-Hernáez P, Reinosa R, Holguín Á. Evolution of SARS-CoV-2 envelope, membrane, nucleocapsid, and spike structural proteins from the beginning of the pandemic to September 2020: a global and regional approach by epidemiological week. *Viruses*, 2021; 13(2): 243-259.

[3] Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews Molecular Cell Biology,* 2022; 23(1): 3-20.

[4] Zhang J, Xiao T, Cai Y, Chen B. Structure of SARS-CoV-2 spike protein. *Current*

Opinion in Virology, 2021; 50:173-182.

[5] Pal M, Berhanu G, Desalegn C, Kandi V. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2): an update. *Cureus*, 2020;12(3): e7423.

[6] Sharma A, Tiwari S, Deb MK, Marty JL. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2): a global pandemic and treatment strategies. *International Journal of Antimicrobial Agents*, 2020; 56(2): 106054.

[7] Babyn PS, Chu WCW, Tsou IYY, Wansaicheong GKL, et al. Severe acute respiratory syndrome (SARS): chest radiographic features in children. *Pediatric Radiology*, 2004; 34(1): 47-58.

[8] Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell*, 2017; 168(6): 960-976.

[9] Mazewski C, Perez RE, Fish EN, Platanias LC. Type I interferon (IFN)-regulated activation of canonical and non-canonical signaling pathways. *Frontiers in Immunology*, 2020; 11: 606456.

[10] Fattahi S, Khalifehzadeh-Esfahani Z, Mohammad-Rezaei M, Mafi S et al. PI3K/Akt/mTOR pathway: a potential target for anti-SARS-CoV-2 therapy. *Immunologic Research*, 2022; 70: 1-7.

[11] Li T. Diagnosis and clinical management of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection: an operational recommendation of Peking Union Medical College Hospital (V2.0) working group of 2019 novel coronavirus, Peking union medical college hospital. *Emerging Microbes & Infections*, 2020; 9(1): 582-585.

[12] Cui N, Zou X, Xu L. Preliminary CT findings of coronavirus disease 2019 (COVID-19). *Clinical Imaging*, 2020; 65: 124-132.

[13] Tok YT, Kuşkucu MA, Erdem H, Sarıbal D, et al. Detection of SARS-CoV-2 RNA in Upper Respiratory Swap Samples by Pooling Method. *Balkan Medical Journal*, 2022; 39(1): 48-54.

[14] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods*, 2001; 25(4): 402-408.

[15] Hosmer Jr DW, Lemeshow S, Sturdivant RX. Applied logistic regression. *John Wiley & Sons.*, 2013; Vol. 398.

[16] Metz CE. Basic principles of ROC analysis. in Seminars in nuclear medicine. Elsevier, 1978.

[17] Jiang Y, Zhao T, Zhou X, Xiang Y, et al. Inflammatory pathways in COVID-19: Mechanism and therapeutic interventions. *MedComm*, 2022; 3.3: e154.

[18] Yang M, Lu Y, Piao W, Jin H. The translational regulation in mTOR pathway. *Biomolecules*, 2022; 12(6): 802.

[19] Tang Y, Kwiatkowski DJ, Henske EP. mTORC1 hyperactivation in lymphangioleiomyomatosis leads to ACE2 upregulation in type II pneumocytes:

implications for COVID-19. *European Respiratory Journal*, 2021; 57(2).

[20] Schreiber KH, Apelo SIA, Yu D, Brinkman JA, et al. A novel rapamycin analog is highly selective for mTORC1 in vivo. *Nature Communications*, 2019; 10(1): 1-12.

[21] Andrade MA, Bork P. HEAT repeats in the Huntington's disease protein. *Nature Genetics*, 1995; 11(2): 115-116.

[22] Yoshimura SH, Hirano T. HEAT repeats–versatile arrays of amphiphilic helices working in crowded environments? *Journal of Cell Science*, 2016; 129(21): 3963-3970.

[23] Mashayekhi-Sardoo H, Hosseinjani H. A new application of mTOR inhibitor drugs as potential therapeutic agents for COVID-19. *Journal of Basic and Clinical Physiology and Pharmacology*, 2022; 33(1): 17-25.

[24] Moni MA, Quinn JMW, Sinmaz N, Summers MA. Gene expression profiling of SARS-CoV-2 infections reveal distinct primary lung cell and systemic immune infection responses that identify pathways relevant in COVID-19 disease. *Briefings in Bioinformatics*, 2021; 22(2): 1324-1337.

[25] Carapito R, Li R, Helms J, Carapito C, et al. Identification of driver genes for critical forms of COVID-19 in a deeply phenotyped young patient cohort. *Science Translational Medicine*, 2022; 14(628): eabj7521.

[26] Bunyavanich S, Do A, Vicencio A. Nasal gene expression of angiotensin-converting enzyme 2 in children and adults. *Jama*, 2020; 323(23): 2427-2429.

[27] Jackson H, Calle IR, Broderick C, Habgood-Coote D, et al. Characterisation of the blood RNA host response underpinning severity in COVID-19 patients. *Scientific Reports*, 2022; 12(1): 1-14.

[28] Zambalde ÉP, Dias TL, Maktura GC, Amorim MR, et al. Increased mTOR signaling and impaired autophagic flux are hallmarks of SARS-CoV-2 infection. *Current Issues in Molecular Biology*, 2022; 45(1): 327-336.

[29] Bolourian A, Mojtahedi Z. Obesity and COVID-19: The mTOR pathway as a possible culprit. *Obesity Reviews*, 2020; 21(9): e13084.

[30] Gordon DE, Jang GM, Bouhaddou M, Xu J, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature*, 2020; 583(7816): 459-468.

[31] Gassen NC, Papies J, Bajaj T, Emanuel J, et al. SARS-CoV-2-mediated dysregulation of metabolism and autophagy uncovers host-targeting antivirals. *Nature Communications*, 2021; 12(1): 3818.

[32] Ancochea J, Izquierdo JL, Group SC-R, Soriano JB. Evidence of gender differences in the diagnosis and management of coronavirus disease 2019 patients: an analysis of electronic health records using natural language processing and machine learning. *Journal of Women's Health*, 2021; 30: 393–404.

[33] Ramírez-Soto MC, Ortega-Cáceres G, Arroyo-Hernández H. Sex differences in

COVID-19 fatality rate and risk of death: an analysis in 73 countries, 2020–2021. *Infezioni Med*, 2021; 29: 402–407.